P8

Evidence of surface structure of the cultured astrocytes and glioblastoma cell culture after exposing β-amyloids (Aβ1-40, Aβ1-42, tau-protein) isolated from the aged human brain with Alzheimer's disease

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The main characteristic traits of Alzheimer's disease (AD) are the violation of the metabolism of amyloid precursor protein (APP), deposition of amyloid-beta (Aβ) plaques and neurofibrillary tangles in the brain tissue, neuronal death and the loss of synapses. All of these signs contribute to cognitive decline in a progressive manner up to total disintegration of memory, speech and visually-spatial properties of the person. Understanding the contribution of these pathological events has been a focus of intensive research study from the last decades. However, our progress in this area has been limited by the difficulties in generating in vitro specific models that recapitulate this pathology. Here we described the nanoscale evaluation of the structural changes of cytoplasmic membrane of macroglial and glioblastoma cell line under the action of various nano-sized pathological forms of Aβ-amyloids, tau-protein which isolated from brain tissue of patients who had died from AD.

For atomic force microscopy (AFM) investigations, we study the rat C6 glioma cell line and mouse EPNT-5 glioblastoma cells collected from Institute of cytology of the Russian Academy of Science (St. Petersburg). All cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin G, 0.1 mg/ml streptomycin (37 °C with 5% CO_2). The isolation of pathological proteins (Aβ-amyloids, tau-protein) was performed according to the protocols of A. Rostagno, J. Ghiso, 2009 [1] and V. M. Lee, J. Wang, 1999 [2]. The treatment of experimental cultures (C6, EPNT-5) β-amyloids, tau-protein was carried out in the logarithmic growth phase by adding 40 μl of purified brain fraction (concentration ~ 0.4 mmol/ml, the dilution steps – 10^{-1}-10^{-5}). The culture flasks were incubated at 37 °C for 48 hours, daily observing the integrity, density of the cell monolayer and morphology. AFM analysis was done at room temperature in tapping mode using Si_3N_4 with a resonant frequency of 190-315 kHz.

AFM investigations of the analyzed cell cultures revealed a considerable range of morphologies of transforming cells as comparison of intact cell cultures (Fig.1 a-f) The untreated cells (C6, EPNT-5) showed a conventional cellular shape with distinct boundaries and centrally located two or three nuclei. When (C6, EPNT-5) cells were exposed to β-amyloids for 12 hours, obvious apoptotic changes, such as aggregation, micronucleated cells and floating cells, were observed. Interestingly, the cell membranes were severely damaged. These changes also included increases in the fluctuation of the surface components of the cell membrane, increase in shrinkage, the absence of pores, formation of giant rounded cells with the loss of lamellopodia.

Analysis of the surface roughness parameters (R_a, R_q) cytoplasmic membranes showed that intact cells differ by a much larger arithmetic average and root-mean-square roughness (R_a = 76 ± 14 nm, R_q = 118 ± 14 nm, $p < 0.01$) compared to with experimental cultures (R_a = 252 ± 15 nm, R_q = 204 ± 14 nm, $p < 0.01$). Thus, it has been shown that Aβ-amyloids affect the cellular cytoplasmic membrane, changing its structure and its spatial organization.

In order to obtain more complete information about the structure of the cytoskeleton, force spectroscopy and measurement of the local values of the Young's modulus were used both in intact astrocytes and in experimental cultures. The force curves were taken at points located along selected lines (10 or 20 points per line). It was shown that on intact astrocytes, the range of values of the Young's modulus had a large scatter (1.4-7.8 kPa), which is typical of most active proliferating eukaryotic cells. At the same time, the Young's modulus subjected to the

transformation with Aβ-amyloids was significantly higher (~17.9 kPa). This is likely to indicate that bundles of fibrils of cells transformed with β-amyloids more closely adhere to their cytoskeleton, which increases the local rigidity of these cells.

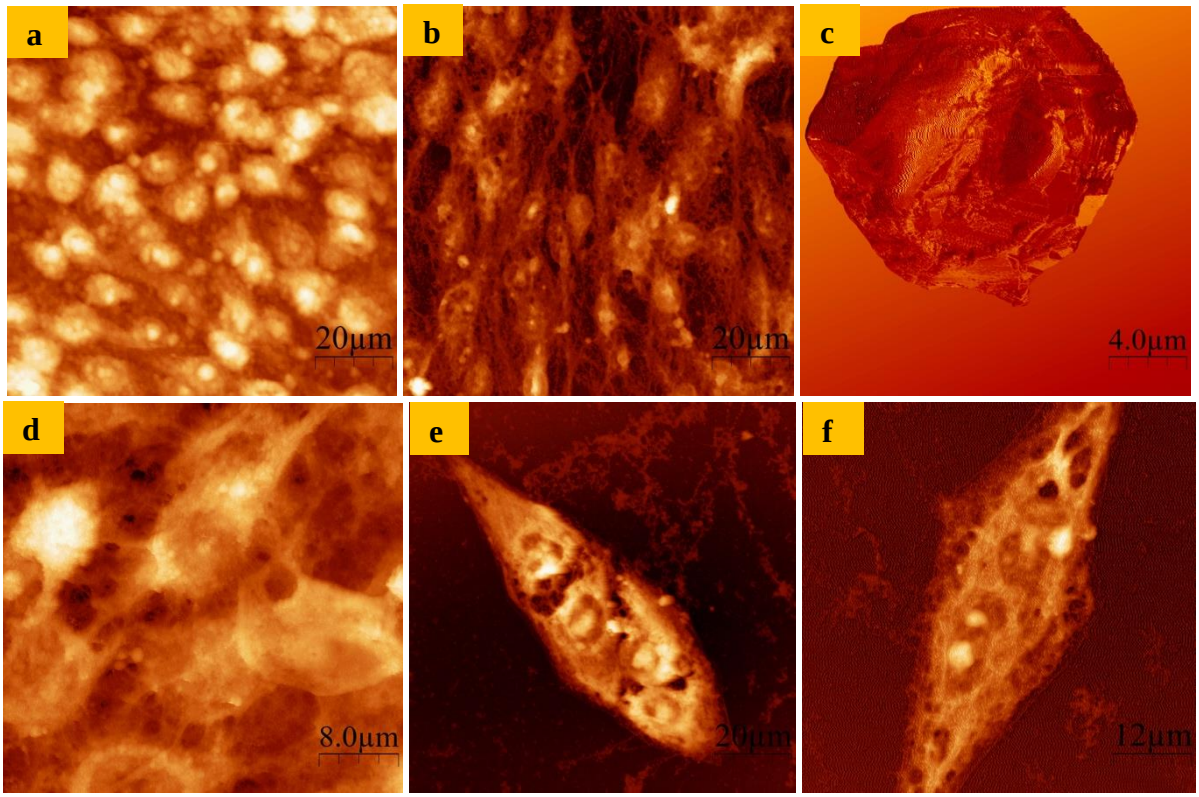

Figure 1. AFM images of topography of the normal (a,d) and treated C6 (b,c), EPNT-5 cell cultures (e, f) with β-amyloids and tau-protein which were isolated from brain tissue of patients with Alzheimer's disease.

Besides, another marker (tau-protein, concentration 0.05 mg/ml) was found to disrupt the cell membrane, not similar to β-amyloids. We revealed more crude morphological changes in astrocytes and glioblastoma (protrusions and loss of filopodia, local fragmentation of the cytoplasmic membrane, the releasing of the contents of the cytoplasm into the intercellular space). Also we have found significant differences in the morphological features between untreated and experimental EPNT-5 cells. We found a very small number of giant cells against the background of destroyed cells, which retained three or four nuclei and well-defined components of the cytoskeleton. These cells are likely to meet specific forms of glioblastomas that contribute to the survival of cancer in the host. However, their role is currently virtually unknown.

1. A. Rostagno, J. Ghiso, *Curr. Protoc. Cell Biol.* **1**, 1 (2009)
2. V.M. Lee, J. Wang, *Methods Enzymol.* **309**, 81 (1999).